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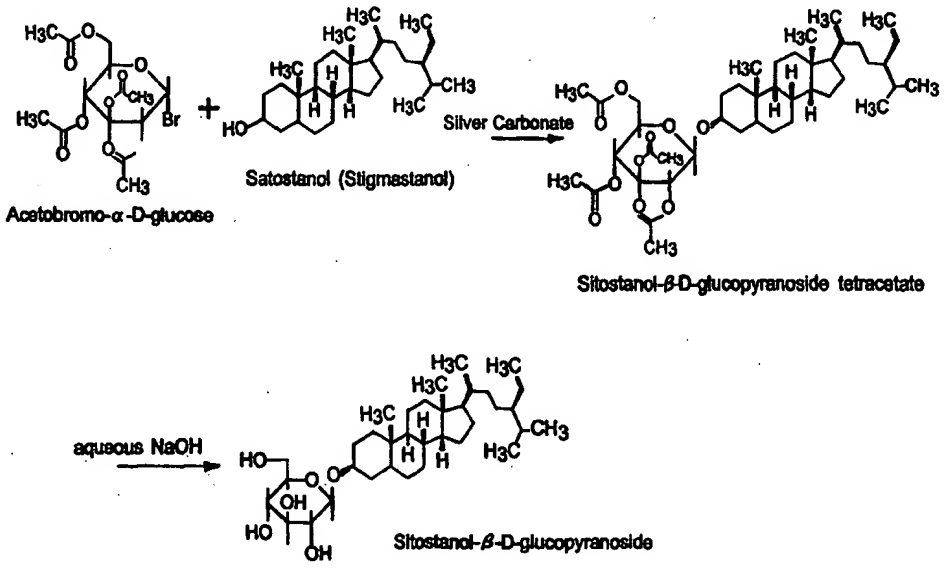

(57) Abstract

A process of preparing sterol glycosides in a form that is easily absorbed through the digestive tract, and methods of treating cholesterolemia by administering a composition which includes at least one glycoside or glycoside ester of a steryl glycoside selected from a group consisting of β-sitosteryl-β-D-glycoside, stigmasteryl-β-D-glycoside and campasteryl-β-D-glycoside and saturated steryl glycosides corresponding thereto.

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READILY ABSORBABLE PHYTOSTEROL GLYCOSIDES TO TREAT HYPERCHOLESTEROLEMIA

Background of the Invention

Phytosterols are components of plants and grains and are contained in small amounts in their cells. About 100 different phytosterols have been isolated from plants. The most abundant by far are (in order) sitosterol, campesterol and stigmasterol. They are structurally very similar to cholesterol except they are alkylated at the 24-position in the side chain.

Phytosterols are natural components of the diet and are consumed in amounts of 100-500 mg/day with US consumption being generally low. (Weirauch, J.L., Gardner, J.M. 1978. Sterol content of foods of plant origin. *J. Am. Diet. Assoc.* 73:39-47) Reportedly, phytosterols themselves are absorbed in relatively small amounts. (Grundy, S. M. and H. Y. I. Mok. 1977. Determination of cholesterol absorption in man by intestinal perfusion. *Journal of Lipid Research* 18:263-271)

The effect of phytosterols on cholesterol absorption has been studied in humans using intestinal intubation where cholesterol is infused and its disappearance is measured in the presence or absence of phytosterols. Using this technique and giving sitosterol in a micellar form (dissolved in monoglyceride), the amount of cholesterol absorbed reportedly was reduced, declining by 79% of the mass of sitosterol infused. (Martin et al., *Lancet*, 2:933-936 (1986) In 2 out of 5 sitosterol infusions cholesterol absorption was reduced to zero indicating an extraordinary potential for efficacy. In 8 clinical trials low density lipoprotein (LDL) cholesterol was lowered 7-33% by sitosterol or its 5-ß-reduced metabolite sitostanol. (Ling, W. H. and P. J. H. Jones. 1995. Dietary phytosterols: A review of metabolism,

benefits and side effects. *Life Sciences* **57**:195-206) Sitostanol reportedly is more effective than sitosterol for cholesterol lowering in animals (Sugano, M., Morioka, H., Ikeda, I. 1977. A comparison of hypocholesterolemic activity of beta-sitosterol and beta-sitostanol in rats. *J. Nutr.* **107**:2011-2019) and has nearly no absorption from the intestine. (Hassan, A.S.,Rampone, A.J. 1979. Intestinal absorption and lymphatic transport of cholesterol and beta-sitostanol in the rat. *J. Lipid Res.* **20**:646-653) Sitostanol given primarily as the oleate ester in margarine reduced serum LDL cholesterol by 14% in a random population sample of 153 individuals with moderate hypercholesterolemia (mean 237 mg/dl) and moderate dietary cholesterol intake of 308-340 mg/day. (Miettinen, T. A., P. Puska, H. Gylling, H. Vanhanen, and E. Vartiainen. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *New England Journal of Medicine* **333**:1308-1312) In the subgroup receiving 2.6 g/day of sitostanol oleate total plasma cholesterol declined from 235 mg/dl (at the upper end of the NCEP "borderline high" classification) to 210 mg/dl (near "ideal") while LDL cholesterol declined 16%.

Miettinen et al. *New England J. Med.*, November 16, 1995, pp 1308-1351 discloses that fatty acid esters of phytosterols reduce cholesterol.

Although 1.8-2.6 g/day sitostanol oleate solubilized in rapeseed oil margarine was highly effective in reducing serum cholesterol, in another trial, 3.0 g/day of unesterified sitostanol suspended (not dissolved) in a small amount of oil was ineffective. (Denke, M. A. 1995. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. *Am. Journ. of Clinical Nutrition* **61**:392-396)

Phytosterols may be given orally as the free sterols in aqueous suspension or as dry powders. However, phytosterols are insoluble in water and poorly soluble in oil and it may take several days to achieve final equilibrium solubility when sitosterol crystals are added to aqueous bile salt micelles. (Armstrong, M. J. and M. C. Carey. 1987.Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. *Journal of Lipid Research* **28**:1144-1155)

Summary of the Invention

The present invention relates to steryl glycosides in a form that is easily absorbed through the digestive tract. The invention also generally features a method of treating cholesterolemia. The composition and method for treating cholesterolemia comprises at least one glycoside or glycoside ester of a steryl glycoside selected from a group consisting of ß-sitosteryl-ß-D-glycoside, stigmasteryl-ß-D-glycoside and campasteryl-ß-D-glycoside and saturated steryl glycosides corresponding thereto, said steryl glycoside being dissolved or dispersed in a solubilizing macromolecule. Dispersion is particularly important because, surprisingly, the dispersed material is absorbed through the intestine at a substantial concentration, enhancing anti-cholesterolemic effect without the need to use substantial amounts of fatty substances as carriers.

Particular useful solubilizing macromolecules include phospholipids and starch, modified starch, alphalized starch, dextrin, sodium starch phosphate, glucose, lactose, monosaccharides, disaccharides, polysaccharides hydroxypropyl cellulose, methyl cellulose, and lecithin. Saturated (e.g., hydrogenated steryl-ß-D-glucoside) or unsaturated steryl glycosides may be used. Particularly useful glycosides include the glucosides, galactosides, maltosides, lactosides or cellobiosides, e.g., ß-D-galactoside, ß-D-maltoside, ß-D-

lactoside or ß-D-cellobioside. The therapeutic may be prepared from a solid residue remaining after removal of water or other solvents from a solution or suspension of said glycoside and the carrier or diluent. Typically, the steryl glycoside has a particle size of 1 - 100 micron. Glycoside esters may be used. These formulations are particularly useful as oral pharmaceutical compositions comprising an effective amount of the steryl glycoside and a pharmaceutically acceptable carrier or diluent.

Phytosterols are not water-soluble and, if they are not absorbed, they may be excreted after ingestion with little or no effect to lower cholesterol. The invention enhances bioavailability of phytosterols by enhancing absorption in the intestine.

The invention also avoids discomfort and other problems associated with oral administration of phyosterols -- e.g,, pure phytosterols pressed into one-gram tablets can create stomach disorders. We concluded that these tablets cannot be readily digested and absorbed and therefore create a discomfort. Unmixed sitosterol powder may appear in stool samples from patients undergoing cholesterol turnover studies where sitosterol was given as a stool marker. According to the invention, sitostanol (for example) is delivered in a more soluble form without using oil or margarine as a vehicle avoiding the substantial disadvantage of administering oil to a patient in need of cholesterol reduction -- giving 3 g/day of sitostanol oleate in oil requires about 30 g oil with 270 calories.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiment and from the claims.

Brief Description of the Figure

Fig. 1 is an outline of a synthesis explained below.

Description of the Preferred Embodiments

We have prepared dispersions of phytosterols and the hydrogenated phytosterols in dispersions of liquids and solids. In addition we have prepared phytosterol derivatives like phytosterol glycosides and glycoside esters. The glycoside moiety enhances their absorption in the intestines and increases their bioavailability.

Suitable sources of sterols include soybeans, wood, and apples. The sterols may be obtained from these sources by known techniques, e.g., by extraction and recrystallization

Glycosylation of the sterols may be achieved by various techniques, e.g., by the general technique described by Vogel, *Tetrahedron Lttrs.* 26:1713 et seq. (1985). Reactive monosaccharide derivatives used for glycosylation may be obtained from Sigma Chemical Co.

Liposomes containing the glycosides may be prepared by techniques generally described below.

Typical dosages according to the invention are from 10-500 mg/75kg patient. This dosage may be formulated in a powder and dispersed in a polymer such as starch as described below. The dispersion is inserted into a standard soft gel capsule.

Various techniques are known to test the dosage in animals and humans.

Examples of Preparation Procedures

Preparation of Hydrogenated Phytosterols

Phytosterols from soybeans containing sitosterol campesterol and stigmasterol (30 gram) was dissolved in 400 ml of ethylacetate and poured into a 600 ml stainless steel pressure vessel. Two grams of palladium on carbon (10% dispersion) was added. The pressure vessel was charged with hydrogen to a pressure of 1000 psi and magnetically stirred. After 2 hours no additional pressure drop was observed. After 24 hours the pressure was released and the content of the vessel was filtered

to remove the catalyst. The solvent was evaporated on a rotary evaporator. The dry product was re-crystallized from hot ethanol two times. A sample dissolved in $CDCl_3$ analyzed by NMR showed the absence of double bonds.

Preparation of Phytosterol Glycosides and Hydrogenated Phytosterol Glycosides

For the preparation of sugar-sterol conjugates, there are a number of reactive monosaccharide derivatives commercially available. Acetobromo-ß-D-glucose (2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyl bromide) was reacted with the soybean sterol mixture of sitosterol, campesterol and stigmasterol to form the ß-D-glucopyranoside. In addition we used the hydrogenated soybean sterol mixture of sitostanol, campestanol and stigmastanol to prepare the saturated sterol glycosides. Other conjugates can be prepared from similar derivatives, also available commercially, like the acetobromo derivatives of galactose, glucouronic acid, maltose, and fucose. A sample reaction is outlined in Fig. 1.

Preparation of Dispersions with Phytosterols, Hydrogenated Phytosterols and their Glycosides.

Dispersions in Lecithin (Phospholipids)

Five gram of soybean sterol was added to 20g of Lecithin. A high-speed stirrer was used to disperse the sterols. The stirrer with sheer action consisting of an outer cylinder and stirring blades rotating inside the cylinder was used. The rotating blades are projecting and pressing the granules against the inside of the cylinder and grind them to smaller particles. The size of the particles can be varied by the duration of the stirring and by increasing or decreasing the rotation of the blades. Particle sizes of 20 - 50 microns were obtained. The particle sizes of the dispersion were determined by

filtration with a Millipore filter. The dispersion was passed through the filter of defined pore sizes of 20 - 50 micron by vacuum suction. All of the dispersed particles passed through the filter indicating that the dispersion contained particle size of less than 50 microns.

Dispersions in solid carriers

The sterol glycoside is dissolved or dispersed by heating in water. Water soluble or dispersible polymer or polymers are added like starch or modified starch includes natural starch obtained from corn, potato or arrowroot, alphalized starch, dextrin. Water soluble starch or cellulose derivatives such as esterified starch (sodium starch phosphate), hydroxypropyl cellulose, methyl cellulose, and the like can also be used.

A typical preparation example:

In 300 ml of water 6 gram of cornstarch was dispersed with a high-speed mixer, 0.6g of soybean sterol-glycoside was added while stirring. The mixtures were dispersed at temperatures of 50°C for 30 minutes. Afterwards the water was removed with a rotary evaporator. The obtained cake was pulverized in a ball mill for 5 hours. To test the particle sizes of the solid dispersion 0.5 gram of the mixture was dispersed in 100 ml of water with a high-speed mixture. The suspension was filtered through a Millipore filter of defined pore size of 20 - 50 micron. No residue was collected on the filter indicating particle sizes were obtained of less then 50 micron.

The complete effect of phytosterols on serum cholesterol may require several months for full expression. See, Miettinen et al., *New Eng. J. Med.* 333:1308-1312 (1995), and Goodman et al. *J. Lipid Res.* 21:699-713 (1980) and clinical trials with respect to

cholesterol lowering are designed accordingly. The total body cholesterol burden is approximately 72 g and compartmental turnover times may be a matter of weeks or even several months.

Inhibition of cholesterol absorption can be determined over a short time period. However, very few clinical trials have actually measured cholesterol absorption because it has required the use of radioactive isotopes and stool collection or gastrointestinal intubation. Non-radioactive cholesterol tracer molecules may be labeled with deuterium and detected by mass spectrometry to measure cholesterol absorption directly, (Lutjohann, D., C. O. Meese,J. R. Crouse,III, and K. von Bergmann. 1993. Evaluation of deuterated cholesterol and deuterated sitostanol (provided by Medical Isotopes, Inc.) for measurement of cholesterol absorption in humans. *Journal of Lipid Research* **34**:1039-1046) and that technique can be used to measure the effect of the invention on absorption.

What is claimed is:

1. A method of treating cholesterolemia by administering a composition which comprises at least one glycoside or glycoside ester of a steryl glycoside selected from a group consisting of ß-sitosteryl-ß-D-glycoside, stigmasteryl-ß-D-glycoside and campasteryl-ß-D-glycoside and saturated steryl glycosides corresponding thereto, said steryl glycoside being dissolved or dispersed in a solubilizing macromolecule.

2. A method according to claim 1 wherein the steryl glycoside is a saturated steryl glycoside.

3. A method according to claim 1 wherein the steryl glycoside is a glucoside, a galactoside, a maltoside, a lactoside or a cellobioside.

4. A method according to claim 2 wherein the steryl glycoside is a hydrogenated steryl-ß-D-glucoside, ß-D-galactoside, ß-D-maltoside, ß-D-lactoside or ß-D-cellobioside.

5. A method according to claim 1 wherein the solubilizing macromolecule is a phospholipid.

6. A method according to claim 1 in which the solubilizing polymer is a carrier or diluent selected from the group consisting of starch, modified starch, alphalized starch, dextrin, sodium starch phosphate, glucose, lactose, monosaccharides, disaccharides, polysaccharides hydroxypropyl cellulose, methyl cellulose, phospholipids and lecithin.

7. The method of claim 5 wherein the steryl glycoside is part of a solid residue remaining after removal of water or other solvents from a solution or suspension of said glycoside and the carrier or diluent.

8. A method according to claim 1 wherein the steryl glycoside has a particle size of 1 - 100 micron.

9. A method according to claim 1 in which the steryl glycoside is a glycoside ester.

10. An oral pharmaceutical composition comprising an effective amount of a saturated steryl glycoside and a pharmaceutically acceptable carrier or diluent.

11. A composition according to claim 10 wherein the glycoside has a particle size of 1 - 100 micron.

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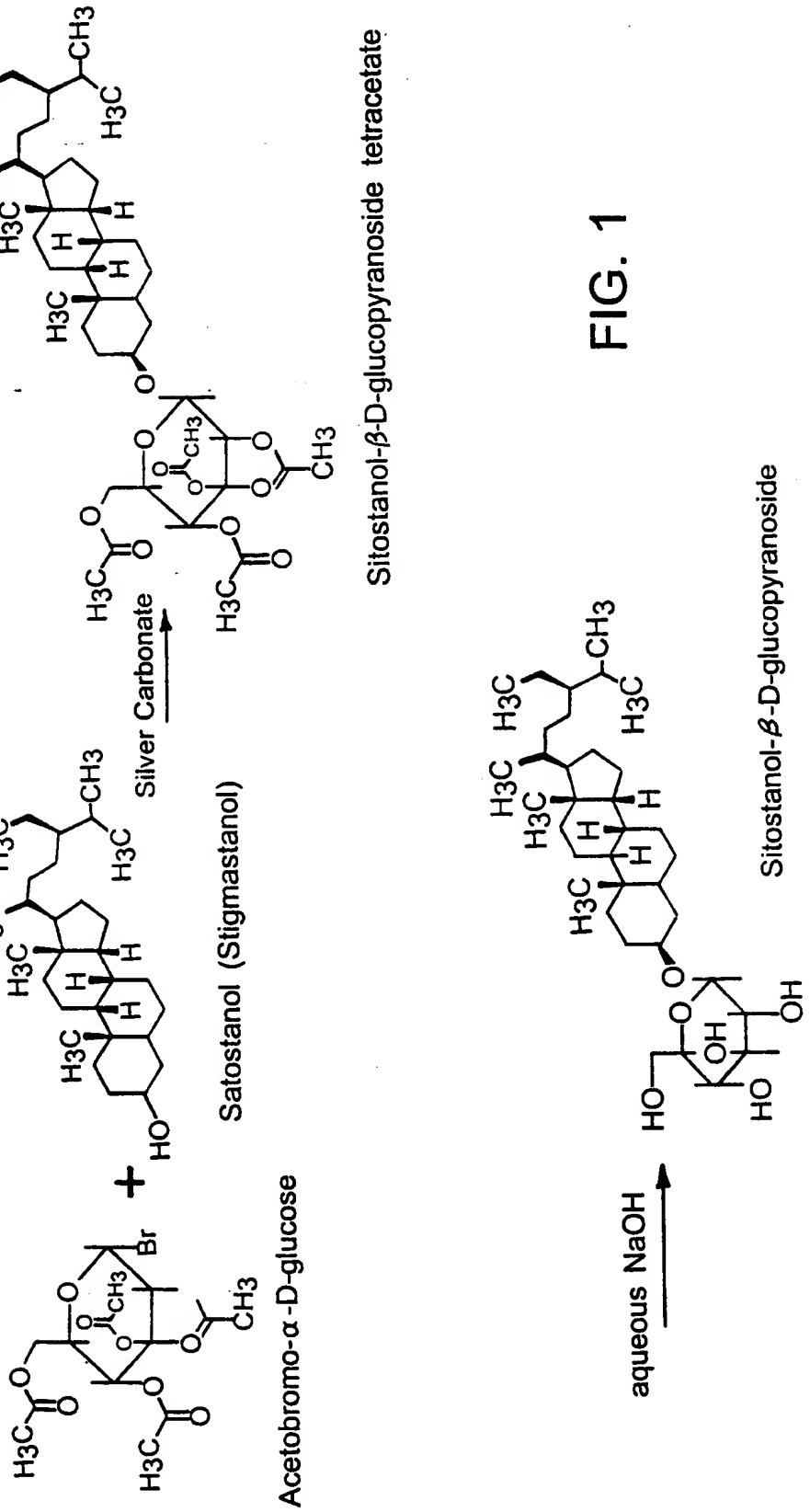
Acetobromo-α-D-glucose
Satostanol (Stigmastanol)
Silver Carbonate
Sitostanol-β-D-glucopyranoside tetracetate
aqueous NaOH
Sitostanol-β-D-glucopyranoside

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/21735

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/705, 9/50, 9/127
US CL :424/450, 499; 514/26, 950; 536/5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450, 499; 514/26, 950; 536/5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, HCAPLUS, WPIDS
search terms: sitosterol, stigmasterol, campesterol, hypercholesterolemia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 4,614,796 A (KAWAMATA ET AL.) 30 September 1986, columns 1-4.	1-7, 9 & 10 --- 8, 11
X -- Y	DE 40 38 385 A1 (HARMSEN ET AL.) 04 June 1992, column 3 and 4.	1-7 & 10 --- 8, 9 & 11
X -- Y	JP 8-231417 A (CHUGAI PHARM CO LTD) 10 September 1996, see entire document.	1-7 & 10 --- 8, 9 & 11
X -- Y	JP 9-135672 A (RYUKAKUSAN KK) 27 May 1997, see entire document.	1-7 & 10 --- 8, 9 & 11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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